

CDK inhibition and cancer therapy

Michelle D Garrett* and Ali Fattaey†

The cell-division cycle is a tightly controlled process that is regulated by the cyclin/CDK family of protein kinase complexes. Stringent control of this process is essential to ensure that DNA synthesis and subsequent mitotic division are accurately and coordinately executed. There is now strong evidence that CDKs, their regulators, and substrates are the targets of genetic alteration in many human cancers. As a result of this, the CDKs have been targeted for drug discovery and a number of small molecule inhibitors of CDKs have been identified.

Addresses

Onyx Pharmaceuticals, 3031 Research Drive, Richmond, California 94806, USA

*e-mail: mgarrett@onyx-pharm.com
†e-mail: afattaey@onyx-pharm.com

Current Opinion in Genetics & Development 1999, **9**:104–111

<http://biomednet.com/elecref/0959437X00900104>

© Elsevier Science Ltd ISSN 0959-437X

Abbreviations

CDK	cyclin-dependent kinase
CKI	CDK inhibitory protein
DMAP	6-dimethyl aminopurine
EGF	epidermal growth factor
NGF	nerve growth factor
RB	retinoblastoma

Introduction

Mammalian cell division is regulated by the timely and coordinated activation of the cyclin-dependent kinase (CDK) family. Regulation of CDK activity occurs at multiple levels, including cyclin synthesis and degradation; activating and inactivating phosphorylation events; CDK inhibitor protein synthesis, binding and degradation; and subcellular localization ([1–5]; see Figure 1). Undoubtedly with the magnitude of research directed at CDKs, further insights into novel mechanisms of their regulation will be revealed. Regulation of CDK activity is essential to the ordered execution of the processes that govern cell growth, complete DNA replication and mitotic transfer of the genome to new daughter cells. To ensure this, surveillance mechanisms function as checkpoints to control cell-cycle progression in case the conditions for advancement have not been met [6,8,9*,10]. As one of their functions, these signaling pathways exert their effects on cell-cycle progression through CDK regulation. Similarly, as part of their function, growth-promoting signal transduction pathways must transmit their effects on cell-cycle progression by modulating CDK activity [11–14]. As with components of these signal transduction pathways that are so often genetically altered in human cancers, it is befitting that CDKs, their regulators, and substrates, are also frequently the targets of genetic lesions, and promote neoplastic transformation [15,16]. The best-characterized case of such alteration is the retinoblastoma (RB) pathway. Under

normal conditions, phosphorylation of pRb by the Cdk4 or Cdk6 enzyme in complex with one of the D-type cyclins are required for $G_1 \rightarrow S$ phase transition. Conversely, pRb's unphosphorylated state is essential for mitotic division cycle exit. Cdk4 and Cdk6 are specifically inhibited by the INK4 small molecular weight CDK inhibitor family. It is noted that alterations in one or another component of this pathway is found in nearly all human cancers [15–17].

Excellent reviews have recently documented the multiple modes of CDK regulation, interactions between CDK regulatory pathways and checkpoint control mechanisms and oncogenic alterations of cell-cycle components. Our attempt here is to illustrate the potential for development of therapeutics to treat human cancers by interfering with cell-cycle progression. Because of the central role that they play in advancing the division cycle, CDKs have been targeted for drug discovery and a number of small molecule compounds have now been identified as CDK inhibitors. These strategies and other targets of intervention within the cell cycle are discussed in our review.

Approaches to CDK inhibition

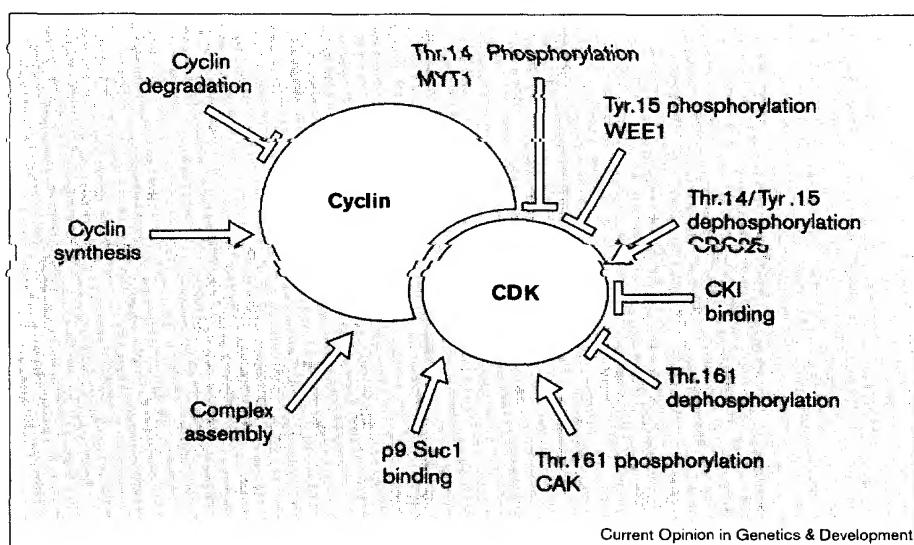
Because of the complex nature of its regulation, modulating CDK activity can be approached via multiple modes for therapeutic intervention. Two basic schemes to inhibit cyclin-dependent kinases are to either directly block the catalytic activity of the CDK, or to target the major regulators of their activity. The most extensively examined of these is catalytic inhibition, which has produced both chemical and peptide/protein based CDK inhibitors. Regulators of CDK activity amenable to therapeutics can encompass: factors involved in the expression and synthesis of the CDK/cyclin subunits or CDK inhibitory proteins, CKIs; proteins that regulate the phosphorylation state of CDKs such as CAK, Cdc25 phosphatases, and the Wee1 and Myt1 kinases; and the machinery involved in proteolytic degradation of the CDK/cyclin complexes or their regulators.

Chemical inhibitors of CDKs

There are six classes of CDK inhibitors that have thus far been characterized: the purine-based compound olomoucine and its analogues, butyrolactone, flavopiridol, staurosporine and the related compound UCN-01, suramin and 9-hydroxyellipticine. Each is either a natural product or derivative of one with a distinct chemical structure. All occupy the ATP-binding pocket of the enzyme and are competitive with ATP. When examining inhibitors that bind to the catalytic site, especially the catalytic site of an enzyme belonging to a large family such as kinases, the issue of specificity becomes a major issue. However, recent experience and success with the development of effective and specific ATP-competitive inhibitors of a number of

Figure 1

Multiple modes of CDK regulation. Regulation of CDK activity occurs at multiple levels, as outlined here. (Thr, threonine; Tyr, tyrosine.) The cdc2 enzyme is used as a reference for sites of phosphorylation (i.e. Thr.14, Tyr.15 and Thr.161). With regard to phosphorylation, the name of the enzyme responsible for a phosphorylation event is given below the event described, for example, threonine 14 phosphorylation is carried out by the MYT1 enzyme.



Current Opinion in Genetics & Development

kinase enzymes has shown that this task is achievable. Olomoucine and its analogues, butyrolactone, and flavopiridol all show strong specificity for CDKs versus a number of unrelated kinases (see Figure 2 for their chemical structures). Staurosporine, UCN-01 and suramin, on the other hand, show no specificity between the CDKs and other kinases such as PKC [18]. In some cases, such as for 9-hydroxyellipticine the inhibitory activity against kinases other than Cdk2 and Cdc2 is unknown [19,20]. It is interesting to note that of these compounds both olomoucine and butyrolactone inhibit Cdc2 and closely related kinases but do not affect the cyclin-D-dependent kinases Cdk4 and Cdk6. Flavopiridol, on the other hand, can inhibit all CDKs tested including Cdk4 [18]. In collaboration with Parke-Davis Pharmaceutical Research, we have recently identified a chemical inhibitor of the Cdk4 and Cdk6 enzymes by high-throughput screening of a large compound library. This ATP-competitive inhibitor is the first to demonstrate great specificity towards these enzymes versus other CDKs and unrelated kinases (MD Garrett, A Fattaey, unpublished data). In the interests of space, we only discuss further the three classes of CDK inhibitors that show strong specificity for CDKs versus other kinases.

Olomoucine, roscovitine, CVT-313 and purvalanol

The first compound identified as a Cdc2 inhibitor was 6-dimethyl aminopurine (DMAP; IC₅₀ = 120 μ M). DMAP was originally synthesized as a puromycin analogue that blocked mitosis in sea urchin embryos without inhibiting protein synthesis [21–23]. Structural analogy searches identified isopentyl adenine as a slightly more potent DMAP analogue with an IC₅₀ of 55 μ M [24]. This strategy lead to the discovery of olomoucine as a potent Cdc2 inhibitor (IC₅₀ = 7 μ M) with specificity for a subset of CDKs [25]. Among 35 kinases tested, olomoucine only inhibited

Cdc2, Cdk2, Cdk5 and MAP kinase in the micromolar range and did not affect Cdk4 or Cdk6 [25]. Crystallization of olomoucine and a related but weaker inhibitor, isopentyl adenine, with Cdk2 revealed that, although both bound in the ATP-binding pocket of the CDK, the adenine side-chain of each lay in a completely different orientation from the adenine group of ATP [26]. Furthermore, the N6 substituent of olomoucine bound outside the conserved region of the binding pocket making contacts with the protein that are not possible for ATP, suggesting that this interaction is most likely responsible for the specificity of olomoucine towards CDKs.

Another substituted purine compound, roscovitine, is a 10-fold more potent Cdc2 inhibitor compared to olomoucine [27,28*]. As evident from its crystal structure with Cdk2, roscovitine binds in a similar orientation as olomoucine, with the N6 substituent also making contacts outside the conserved binding domain affirming the likelihood that this interaction provides the specificity of this compound class for CDKs [28*]. A related compound, CVT-313, has similar inhibitory activity against CDKs as roscovitine and will block neointimal formation in a rat carotid artery restenosis model [29*].

The most recent addition to these purine-based structures is a group of compounds that were identified in a screen of trisubstituted purine combinatorial libraries designed for Cdk2 inhibition [30**]. The best of these *in vitro* is purvalanol B which shows a thousand-fold increase in potency against Cdk2/Cyclin A when compared to olomoucine. Less potent but more membrane-permeable are purvalanol A (IC₅₀ Cdk2/cyclin A = 70 nM) and compound 52. Crystallographic analysis of purvalanol B with Cdk2 reveals that binding into the ATP-binding pocket resembles that of the other substituted purines olomoucine and

クレスト9は支持アーム9bに沿い、バツクレスト9を後方へ転倒した時は椅子8の奥行方向へ、前方へ転倒した時は高さ方向へ位置調節可能である。

椅子8の前方、旋回床7上に平行2本の脚10、10が立設され、該脚10、10の上端に水平格状のハンドレール11が固設され、脚10、10の中間部に架設された横部材10aにニーパッド12が配設され、脚10、10の上部には安全ベルト13の両端が取付けられている。安全ベルト13は端部に穿設された複数の調節孔13aを脚10の突起10bに差換えることによつて長さの調節が可能である。

脚10、10の上部においてハンドレール11の枠内に操作ユニット14が配設されている。該操作ユニット14には例えば走行用レバー14a、旋回用レバー14b、ブレーキ操作ボタン14c、ブレーキ解除ボタン14d、電源表示ランプ14e、電源スイッチ14f、バッテリ表示メータ14g、スピード切換スイッチ14hを備える。そして電源スイッチ14fをオンにし走行用レバー14aを前

傾、後傾、右傾、左傾することによつて台車1は前進、後進、右転向、左転向し、スピード切換スイッチ14hを切換えることによつてスピードが高低二段に切換えられる。又旋回用レバー14bを右傾又は左傾することによつて旋回床7は右方又は左方へ旋回する。旋回床7の旋回中旋回用レバー14bを中立に戻せば旋回床7は任意の旋回位置に停止する。この旋回床7の旋回は台車1内に備えた作動子15に連動され、旋回床7が台車1の前後方向軸線を中心として右方又は左方へ90度旋回した時作動子15が右側又は左側のリミットスイッチ16又は17に接触し、旋回床7の旋回を自動停止させる。ここで旋回用レバー14bを逆方向へ傾倒すれば旋回床7は逆方向へ旋回し、旋回床7が正面に戻つた時作動子15は正面のリミットスイッチ18を作動させて旋回床7を一時停止させる。そのまま旋回用レバー14bを同方向へ倒したままであれば旋回床7は一時停止後再び同方向への旋回を継続する。

ハンドレール11の上面にはテーブル19がそ

の下面に固着されたグリップ19a、19bにて着脱自在に取付けられる。

〔作用〕

次に上記実施例の作用について説明する。使用者はまず自身の体格に合わせて椅子8の脚部8aを伸縮した後ノープボルト8bを締付けて椅子8の高さを固定し、又バツクレスト9を支持アーム9bに沿つて摺動しノープボルト9fを支持アーム9bの調節孔9gに差込んで締付け適当位置にバツクレスト9を固定し、バツクレスト9を後方へ転倒して背凭れとシート8cに椅子する。そして操作ユニット14の電源スイッチ14fをオンにし、走行用レバー14aを前後左右方向に傾動することによつて該電動車椅子は前後左右任意の方向へ移動することができる。この時スピード切換スイッチ14hを切換えることによつて高低二段のスピードが選択できる。又走行中あるいは停止中の何れの場合にも旋回用レバー14bを右傾又は左傾して椅子8の向きを台車1の前後方向軸線を中心として左右へ最大90度まで旋回させることができる。

できる。

立位が必要の場合使用者は自力で立上がり、手でバツクレスト9を前方へ転倒し、臀部をバツクレスト9に当てて体重の一部をニーパッドに託すると共に膝部をニーパッド12に当て、更に安全ベルト13を腰部に巻回してバツクレスト9から身体がずり落ちるのを防ぐ。立位時の運転操作も椅子時の場合と同様である。

上記のごとくして自力で立位をとることが可能だが長時間立位姿勢を維持することが困難な下肢障害者は、椅子位又は立位にあつて作業の目的の場所へ移動し作業位置に正対あるいは側対して作業を遂行し、又必要により旋回あるいは横移動を行ない、更にテーブル19上に物品を載せ運搬する等して作業領域を拡大することができる。そして下肢障害者が立位をとつた時、体重の一部がバツクレスト9に支持されると共にニーパッド12と安全ベルト13によつて身体が安定に保持されるため、長時間立位姿勢を維持することが可能となる。

八. 発明の効果

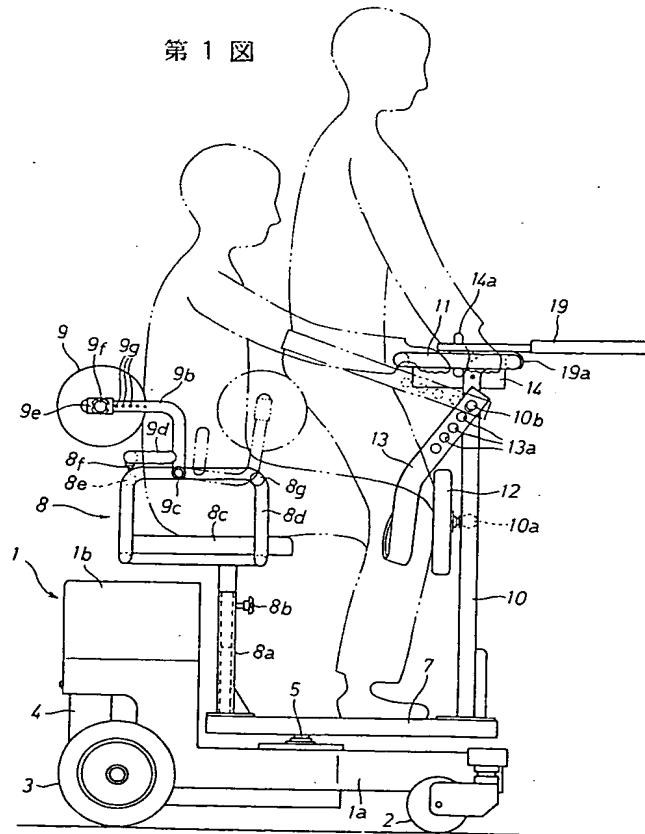
以上述べたごとく本発明に係る立位補助電動車椅子では、従来の自力で椅坐位から立位をとるのが困難な比較的下肢障害度の大な障害者を対象とした電動車椅子に較べ、椅坐位から立位へ体位の変換を介助する助力装置を省くため構造が簡単で軽量小型となり狭い場所での椅坐位から立位に至る作業性を向上することができる。そして廉価に提供できるので自力で立位をとることが可能な比較的軽度の下肢障害者への普及に役立ち、当該障害者の雇用の促進に寄与し得るものである。

4. 図面の簡単な説明

添付図面は本発明の一実施例を示し、第1図は側面図、第2図は平面図である。

1 … 台車、4 … 電動機、7 … 旋回床、8 … 椅子、9 … バックレスト、9b … 支持アーム、9e … 摺動部材、9f … ノブボルト、9g … 調節孔、10 … 脚、11 … ハンドレール、12 … ニーパッド、13 … 安全ベルト、14 … 操作ユニット、19 … テープル。

第1図



第2図

